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REMARKS

A. Request for Reconsideration

Applicant has carefully considered the matters raised by the Examiner in the outstanding Office Action but remains of the position that patentable subject matter is present. Applicant respectfully requests reconsideration of the Examiner's position based on the amendments to the claims and the following remarks.

B. The Invention

The present invention is directed to a process for the separation, identification and quantification of individual microbes in a mixture of microbes using electrokinetic separation systems.

In order to get a kinetic separation of different microorganisms from each other, the applicant discovered that three conditions have to be present SIMULTANEOUSLY in a microfluidic system: 1) electroosmatic flow of the bulk solution, 2) opposite (countercurrent) movement of the microorganism(s) usually due to electrophoresis toward the anode; 3) a very dilute polymer that is not sufficiently concentrated to form an entangled polymer or molecular sieve of any type. Only with these conditions will the different microorganisms sort out, focus and aggregate with their own kind, at different

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rates, and hence they form an aggregated purified particle at different times.

The first two conditions are present in typical electrophoresis. One of the novel aspects of the invention is using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis. The unexpected effect resulted from the adding a dilute hydrophilic neutral polymer in the present invention is evident from the sharp peaks in an electropherogram.

C. Claim Status and Amendments

Claims 1, 3-6, 8-13, 15, 17 and 22-30 are presented for further prosecution. Claims 18-21 have been withdrawn from consideration.

Claims 1, 6, 10, 15, and claim 30 have been amended by changing "focus(es)" into "separate(s)". The support of this amendments can be seen from the last paragraph of page 14 and the discussion in Example 3, in particular the last paragraph of page 24, where it discloses the separation of microbes, one from another by adding of dilute polymers in the running buffer.

There is no new matter added in these amendments.

D. The Office Action

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1. Independent claims 1 6, 10, 15 and 30 and their dependent claims as currently amended are clear of 35 USC 112 rejections

Regarding the USC 112 rejection on "focus(es)", independent claims 1 6, 10, 15 and 30 have been amended to recite that the microbes/cells are separated.

As for USC 112 rejection on "neutral" polymer, the applicant respectfully submit that all the polymers numerated in the specification and claims are neutral polymers (4th paragraph of page 14). In the examiner's citation, U.S. Patent No. 7,033,474 B1 col. 18:33-38 mislabeled polyacrylamide as charged polymer, see the enclosed reference *Pefferkorn* on polyacrylamide; and U.S. Patent No. 6,558,945 B1 col. 4:33-43 do not say the cited polymer are charged. In fact, it is well known, that the polymers numerated in 4th paragraph of page 14 are neutral (See enclosed *Horkay* article on neutral hydrogels). It is a permissible inductive clarification of the type of polymers by using "neutral" in these claims.

In the present invention, the diluted water soluble neutral polymer in the running buffer exerts a focusing effect by

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different mechanism from conventional usage of concentrated, charged polymers in the electrophoresis methods.

It is respectfully submitted that claims 1, 6, 10, 15, and 30, along with their dependent claims 3-5, 8-9, 11-13, 17 and 22-25 comply with the writer description requirement of USC 112 1st paragraph and are therefore definite.

2. Prior Art Rejection under USC 103

i. Rejections on Claims 1, 3, 4, and 22

Claims 1, 3, 4, and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ebersole et al. in view of Johnson et al., the CAPLUS abstract of Barkas et al., Silman, Catsimpoolas, the CAPLUS abstract of Jenkins et al., Grant et al., .and Van Alstine for the obviousness of using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis.

The applicant wishes to bring the following points to the examiner's attention:

1) Barkas, Silman and Catsimpoolas relate to gel electrophoresis, where the polymer gel is a stationary phase in the separation path. The do not teach the use

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of dilute water-soluble polymer in the running buffer of capillary electrophoresis.

2) Johnson's using of polymer is to treat the DNA samples before electrokinetic loading (see col. 6, lines 6-12). Johnson introduces polymer solution into DNA sample, not in the "moving fluid", therefore there is no teaching from Johnson on using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis.

3) Grant uses polymers in **stationary fluid** for Zeta **potential measuring** (see last paragraph, page 18 of Grant). There is no concerns of using a dilute water soluble polymer to **separate microbes** in the **moving fluid** of capillary electrophoresis.

4) Jenkins uses methyl cellulose in CIEF. However, claims 1, 3, 4, and 22 relate to electrophoresis, not capillary isoelectric focusing (CIEF). As the examiner has recognized, using a dilute water soluble polymer to separate microbes in the **moving fluid** of capillary electrophoresis contradicts the necessary stationary focusing phase of CIEF, Jenkins can not teach using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis.

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5) Van Alstine is a totally different technology. It uses PEG **bond to an affinity compound** for separation. This is an affinity electrophoresis separation in which the affinity "tag" is the separating molecule, not the polymer, therefore, Van Alstine does not teaching using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis.

In view of the above fundamental differences, these cited references do not teach, suggest or give any motivation for using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis. Therefore, the combination of these references with Ebersole does not render the claims 1, 3, 4, and 22 obvious.

ii. Rejections on claim 5

Claim 5 is rejected as being unpatentable over Ebersole in view of Johnson, Barkas, Silman, Catsimpoolas, Jenkins, Grant, .and Van Alstine and further in view of the first page of "Streptococcus pyogenes" article.

By a similar argument as (i), the combination of the above references does not teach the critical feature of the present

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invention: using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis. Thus, Claim 5 are not rendered obvious by these references.

iii. Rejections on claims 6, 8-13, and 22-24

Claims 6, 8-13, and 22-24 are rejected as being obvious by Durr et al. (US 5,723,031) in view of Johnson, Barkas, Silman, Catsimpoolas, Jenkins, Grant, .and Van Alstine.

Here, again, the critical feature of the present invention: using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis are not disclosed or suggested. Thus, Claims 6, 8-13, and 22-24 are not rendered obvious by these references.

iv. Rejections on claims 15-17 and 25

Claims 15-17 and 25 are rejected over McCormick et al. (US 6,613,211 B1) in view of Johnson, Grant, Jenkins and Van Alstine.

Once again, the applicant submitted the critical feature of the present invention: using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis are not disclosed or suggested. Thus, Claims 15-17 and 25 are not rendered obvious by these references.

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It should be emphasized that, using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis is conceptually unique, it is not a matter of optimizing the sieving effect of the polymer. The present invention requires a very dilute polymer - such as 0.0125% in example 1, that is diluted to where one dissolved polymer molecule does not contact another not sufficiently concentrated to form an entangled polymer or molecular sieve of any type. Note that the polymer is so dilute that it may or may not weakly/dynamically interact with cells - but it cannot form any physical barrier. Only with these dilute water soluble neutral polymers, will the different microorganisms sort out, focus and aggregate with their own kind, at different rates, and hence they form an aggregated purified particle at different times.

The applicant has conducted a series of experiment to demonstrate the unexpected result of the using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis. A declaration by the inventor on the testing results will follow shortly.

The testing data shows, 1) if a gel is used in our capillary (of any kind) there is no movement of the injected microbe. They never came through the detector. The capillary

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is clogged. 2) If a more conventional solution of a neutral polymer, such as that used in DVA separations (see Kao, 6558945) (and Johnson EP0773252A2), the injected microbes do not separate from each other, nor do the microbes focus. Rather the unseparated injection plug of microbes is carried through the detector in a state similar to how it was injected. 3) With a neutral polymer that is dilute as in the present invention (i.e., ideally dilute where one dissolved polymer molecule does not contact another) the individual microorganisms be separated from each other as sharp, well separated peaks.

The unexpected result demonstrates the inventive step of the present invention. Therefore using a dilute water soluble polymer to separate microbes in the running buffer of capillary electrophoresis is not obvious to the skilled of art.

vi. Rejections on claims 26 and 30

Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Fuhr. Claim 30 is rejected over Fuhr in view of Johnson, Grant, Jenkins and Van Alstine.

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Fuhr is the primary reference and the only reference that had been cited to teach isoelectric focusing step of the claims 26, and 30. The applicant wishes to point out:

1) Fuhr uses an external field (See col. 5, lines 38-40); CIEF uses an "internal" field with direct electrical contact with the buffer and with current flowing in the separation channel.

The present invention is an electrokinetic separation method. An electrokinetic method means that solvent flows due to the movement of hydrated ions (usually cations) which electrophorese in a direct current electrical field. Note there is direct electrical contact with the solution and a current. In Fuhr's FFF method there is no electrokinetic movement of the solvent. Furthermore, there is no direct electrical contact with the separation solution. There is no electrophoresis and no electrosomatic flow as the required condition for the present invention.

2). In Fuhr, the external field is perpendicular to the channel and the flow direction (see Fig. 3); while in CIEF, the current and field are parallel to the channel. Fuhr uses the

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plates of a capacitor to produce an electric field at 90° to the flow. This pushes the cells against the wall

In fact, the external perpendicular field in Fuhr is totally uncompatible with the "internal" parallel electric field so that it can not generate a pH gradient along the capillary channel, which is the essence of CIEF. Therefore, although Fuhr uses the term "isoelectric focusing", Fuhr is not the conventional one used in the present invention.

Applicants respectfully submit that Fuhr is in a different technology from the conventional capillary isoelectric focusing used in Claims 26 and 30 and it doesn't teach the elements of the present invention. It is therefore submitted that claims 26 and 30 of the present invention is patentable over Fuhr and its combination with other references.

E. Conclusion

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance and such action is respectfully requested. Should any extensions of time or fees be necessary in order to maintain this Application in pending

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condition, appropriate requests are hereby made and authorization is given to debit Account # 02-2275.

Respectfully submitted,

LUCAS & MERCANTI, LLP

By: Donald C Lucas

Donald C. Lucas, 31,275
Attorney for Applicant(s)
475 Park Avenue South, 15th Floor
New York, NY 10016
Tel. # 212-661-8000

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